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Lactobacillus rhamnosus GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production

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ABSTRACT

Recently, a probiotic *Lactobacillus rhamnosus* GG (LGG) has shown several beneficial effects, including improved insulin sensitivity. To clarify the mechanism underlying the insulin-sensitizing effect of LGG, mice were orally administrated with LGG for 13 weeks, and their body weight, insulin sensitivity, and expression of genes related to glucose and lipid metabolism were examined. LGG-treated mice showed attenuated weight gain and enhanced insulin sensitivity in high fat diet group, while no change was observed in normal diet-fed group. The expression of fatty acid oxidative genes in the liver was increased and gluconeogenic genes were decreased. GLUT4 mRNA expression in skeletal muscle and adiponectin production in adipose tissue were significantly increased. This was corroborated with the increased activation of AMPK in skeletal muscle and adipose tissue. Taken together, these results indicate that LGG treatment improves insulin sensitivity and reduces lipid accumulation by stimulating adiponectin secretion and consequent activation of AMPK.

1. Introduction

Metabolic syndrome, which referring obesity, dyslipidemia, and insulin resistance, is a multiplex risk factor for coronary artery disease and type II diabetes. Several kinds of drugs are available for diabetic patients, but many of them commonly share a problem of weight gain. Limited types of drug, such as metformin, are known to not cause weight gain, but have been found to have other negative side effects [1,2]. As the diseases caused by metabolic syndrome are closely linked to overweight or obesity [3], it is required to develop novel therapies which are able to prevent weight gain and sustain normal blood glucose levels at the same time.

Probiotics are defined as microorganisms which exert health benefits to the host. Since it has been discovered that gut microbiota is closely related to metabolic states of the host [4–6], many studies have been performed to ameliorate metabolic diseases through probiotics treatment [7–10]. However, the precise mechanism through which probiotics act on the host metabolic state remains to be elucidated.

Of the probiotics, *Lactobacillus rhamnosus* GG (LGG) has been intensively studied and showed several beneficial effects such as prevention of diarrhea [11], atopic diseases [12], and intestinal inflammation [13]. Recently, a few reports suggest that LGG also has an anti-hyperglycemic effect in diabetic animal models. Tabuchi et al. [14] reported that LGG treatment lowered the level of glycated hemoglobin and improved glucose tolerance by stimulating insulin secretion in streptozotocin-induced diabetic rats. Another study performed by Honda et al. [15] also found that LGG treatment enhanced insulin sensitivity in KK-Ay mice. In spite of these apparent anti-diabetic effects of LGG, questions still remain in elucidation of the mechanism underlying the effects. Besides, it is also required to examine whether the effects are associated with any potential side effect of weight gain. In this study, we observed that LGG treatment enhanced insulin sensitivity with reducing weight gain in high fat diet-fed condition, and diminished lipid accumulation in the liver and mesenteric adipose tissue. These results demonstrate that LGG has a beneficial effect on improvement of metabolic syndrome through an augmentation of adiponectin production which results in AMP-activated protein kinase (AMPK) activation.

2. Materials and methods

2.1. Animals

Seven–weeks–old male C57BL/6J mice were purchased from Hyochang Bioscience (Daegu, Korea) and fed a standard chow diet (AIN-93G, Feedlab, Guri, Korea) for a week to stabilize all metabolic conditions. Mice were maintained on a 12 h dark/light cycle at a constant temperature of 22 ± 1 °C and humidity of 55 ± 10%. After stabilization, mice were divided into 2 diet groups, of which the normal diet (ND) group was fed a standard chow diet and the high-fat diet (HFD) group was fed a high-diet diet (D12492, Re-

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search Diets Inc., NJ). The caloric contributions (% protein: % carbohydrate: % fat; kcal/g) are (chow, 20:64:16, 4.0 kcal/g) and (HFD, 20:20:60, 5.24 kcal/g). Each group was divided into 2 subgroups, LGG-treated and control group, which received a daily dose of LGG (1×10^8 CFU per mouse) and PBS orally for 13 weeks, respectively.

Mice were fasted for 4 h and sacrificed, and tissues of liver, epididymal fat, mesenteric fat, and quadriceps muscle were rapidly excised, snap-frozen in liquid nitrogen, and stored at −75 °C until processed for experiments.

2.2. Glucose tolerance test

After 10-week of LGG treatment, mice were fasted for 16 h and followed by intraperitoneal injection of glucose (2 g/kg). Blood samples were obtained by tail-bleeding and blood glucose level was checked by Accu-Check Go (Roche Diagnostics GmbH, Germany) 0, 10, 20, 30, 60, 90, and 120 min after glucose injection.

2.3. Immunoblotting

Frozen tissues were homogenized and protein-extracted as described previously [16]. Briefly, the supernatants obtained by centrifugation of tissue homogenates were separated by 10% SDS–PAGE and transferred to PDVF membranes. After blocking with 5% non-fat milk in TBS–T, the membranes were incubated with the specific antibodies, including anti-(phospho) acetyl–CoA carboxylase (ACC), anti-(phospho) AMP–activated protein kinase (Cell signaling technology, Beverly, MA), and anti-adiponectin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The proteins were visualized by enhanced chemiluminescence using horseradish peroxidase–conjugated anti-rabbit IgG.

2.4. Real-time RT PCR

Total RNA extraction, reverse transcription, and quantitative PCR were performed as described previously [16]. Briefly, total RNA were extracted from tissues with TRI reagent (Molecular Research Center, Cincinnati, OH). cDNA was obtained by reverse transcription of total RNA with ImProm-II™ Reverse Transcriptase (Promega, Madison, WI). Real-time PCR analysis was performed with HotStart-IT SYBR Green qPCR Master Mix (Affymetrix, Santa Clara, CA) and gene-specific forward and reverse primers on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA). Quantification of gene transcripts for acyl–CoA oxidase (ACOX), adiponectin, carnitine palmitoyltransferase 1 (CPT1), glucose transporter 1 (GLUT1), glucose transporter 4 (GLUT4), glucose 6-phosphatase (G6Pase), phosphoenol pyruvate carboxykinase (PEPCK), and peroxisome proliferator-activated receptor-alpha (PPAR-α) was completed using gene-specific primers. Results were expressed as mean ± S.D. after normalizing to expression of GAPDH gene using the $\Delta\Delta Ct$ method.

2.5. Oil Red O staining

Liver samples were fixed with 4% paraformaldehyde in PBS and dehydrated in 30% sucrose PBS at room temperature. Tissues were then immersed in Optimal Cutting Temperature (OCT) solution on dry ice and cut at 10 μm. Cryosections were washed with 60% isopropanol and stained with 0.5% Oil Red O in 60% isopropanol for 15 min. After washing with 60% isopropanol, sections were counterstained with alum hematoxylin, washed with tap water, and mounted with glycerin jelly. Sections were visualized under a Olympus CK40 microscope (Olympus, Tokyo, Japan) at × 400.

2.6. Statistical analysis

Numerical data were presented as means ± S.D. Differences between groups were assessed by Student's *t*-test. P values < 0.05 were considered as statistically significant.

3. Results

3.1. Effects of LGG treatment on glucose tolerance and weight gain

During 13 week, LGG-treated mice gained less weight compared to control mice under high-fat diet (HFD)-fed condition (Fig. 1A). We observed that the weights of the liver and mesenteric adipose tissue were significantly lower in LGG-treated than control mice (Table 1). To examine whether LGG treatment enhances insulin sensitivity, we measured glucose tolerance in mice of both groups. In normal diet (ND)-fed mice, no significant difference was observed between LGG-treated and control groups. However, LGG-treated mice on HFD showed significant enhancement of glucose tolerance compared to control group at 10 weeks of treatment (Fig. 1B). We next performed Oil Red O staining with the liver samples, based on the result of lower liver weight in LGG-treated HFD-fed mice, to confirm the protective effect of LGG from diet-induced hepatic steatosis. HFD feeding caused a remarkable increase of lipid droplet accumulation in the liver as revealed by Oil Red O staining (Fig. 2). Interestingly, the lipid deposition in the liver of LGG-treated HFD-fed mice decreased significantly as compared to controls, whereas it was not different between two groups under ND-fed condition.

3.2. Effects of LGG treatment on mRNA expression of genes involved in gluconeogenesis and lipid oxidation, adiponectin, and GLUT4

To elucidate the mechanism of enhanced glucose tolerance and reduced weight gain by LGG treatment, we analyzed the mRNA level of genes involved in glucose and lipid metabolism in liver, white adipose tissue, and skeletal muscle. In the expression of gluconeogenic genes, glucose-6 phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) in the liver of LGG-treated HFD-fed mice were decreased compared to control group (Fig. 3A). LGG treatment in HFD-fed mice also increased the expression of genes involved in fatty acid oxidation, such as PPAR-α, CPT1, and ACOX in the liver. In mesenteric adipose tissue, a marked increase in PPAR-α, and slight but insignificant increases in CPT1 and ACOX expression, was observed (Fig. 3B). There was also a significant decrease in SREBP1c expression, which was accompanied with slight decreases in ACC and FAS expression. Surprisingly, in epididymal adipose tissue, adiponectin mRNA expression was significantly increased, whereas expression levels of genes involved in glucose transport (Fig. 3C) and fatty acid oxidation were not altered (data not shown). In skeletal muscle, GLUT4 mRNA expression was increased and this was accompanied with increase of CPT1 and ACOX expression (Fig. 3D). These results demonstrate that the enhancement of insulin sensitivity with reduced adiposity by LGG treatment is at least in part associated with increased expressions of adiponectin, GLUT4, and lipid oxidative genes in responsible tissues, such as adipose tissue, skeletal muscle and the liver.

3.3. Effects of LGG treatment on serum adiponectin level and AMPK phosphorylation

To corroborate with the increase of adiponectin mRNA expression, we further investigated the protein level of adiponectin in serum. Western blot results showed a marked increase of adipo-